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RAPID PUBLICATION

LINKAGE ANALYSIS IN A FAMILY WITH THE OPITZ GBBB SYNDROME REFINES THE LOCATION OF THE GENE IN Xp22 TO A 4 cM REGION.

Melanie May, Sara Huston, R. Sid Wilroy and Charles Schwartz

Center for Molecular Studies, J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, S.C. (M.M., C.S.)[1]; Department of Biological Sciences, Clemson University, Clemson, S.C. (S.H., C.S.); Section of Genetics, Department of Pediatrics, University of Tennessee, Memphis, Tennessee (R.S.W.)

The Opitz GBBB syndrome (OS) is characterized in part by widely spaced inner ocular canthi and hypospadias. Recently, linkage analysis showed that the gene for the X-linked form to be located in an 18 cM region spanning Xp22. We have now conducted linkage analysis in a family previously published as having the BBB syndrome and found tight linkage to DXS7104 (Z=3.3, θ=0.0). Our data narrows the candidate region to 4 cM and should facilitate the identification and characterization of one of the genes involved in midline development.

KEY WORDS: Opitz syndrome, GBBB syndrome, linkage, Xp22

INTRODUCTION

The BBB syndrome was first reported independently in 1969 by Opitz et al. [1969a] and Christian et al., [1969]. The syndrome is characterized in part by telecanthus and hypospadias in males with variable degrees of telecanthus in females. Another entity, the G syndrome (Opitz-Frias syndrome) [Opitz et al., 1969b], is characterized by telecanthus, hypospadias, cleft lip and palate, and laryngotracheoesophageal defects. In 1978, Cordero and Holmes reported families in which both syndromes segregated. Additional evidence that the two syndromes represented the same condition was provided by Opitz [1987]. They reported a boy in one of the original BBB families who had the G syndrome. As a result, the syndrome became known as the Opitz GBBB syndrome (OS, MIN 143410) [Neri and Cappa, 1988].

In an evaluation of 12 families, Stevens and Wilroy [1988] suggested that the BBB syndrome was most likely X-linked since males were more severely affected than females and they found no male-to-male transmission. However, the authors mentioned that autosomal dominant inheritance with male limitation was possible since there had been some reports of male-to-male transmission [Stoll et al., 1978; Farndon and Donnai, 1983]. Recently, linkage data indicated that the disease is indeed heterogeneous, with a locus on Xp22 and a second one on 22q11.2 [Robin et al., 1995]. Confirmation of the latter localization has come in the form of three cytogenetic observations of deletions of 22q11.2 associated with the appearance of Opitz GBBB syndrome manifestations [McDonald-McGinn et al., 1995; Fryburg et al., 1996; Lacassie and Arriaza, 1996]. A second autosomal locus may be in distal 13q based on a report of a child with clinical findings consistent with OS and a deletion breakpoint at 13q32.3 [Urioste et al., 1995].

We have established linkage to DXS7104 in Xp22.2 in one of the families reported by Stevens and Wilroy [1988] as having the BBB syndrome. Our findings

[1] Address reprint requests to Charles Schwartz, Ph.D., J. C. Self Research Institute, One Gregor Mendel Circle, Greenwood, S. C. 29646.

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confirm the earlier report of Robin et al., [1995] which placed the X-linked form of the disorder in Xp22. Furthermore, by combining our results with those of Robin et al., [1995] we are able to narrow the candidate region to a 4 cM area flanked by markers DXS1043 (distal) and DXS987 (proximal). This should reduce the effort needed to identify and characterize the gene responsible for OS.

MATERIALS AND METHODS

Clinical Material

Kindred K7055 was previously published as family 2 in the paper by Stevens and Wilroy [1988]. Additional relatives were available for this study and an updated version of the pedigree is given in Fig. 1. All clinical studies in K7055 were conducted by one of us (R.S.W.).

Microsatellite Analysis

The conditions used to generate the specific dinucleotide polymorphisms were as given in Nelson et al., [1995] or as available through GENBANK. The forward primers were synthesized and labeled with fluorescein amidite (FluorePrime, Pharmacia) using a Beckman 1000 DNA synthesizer and desalted through Sephadex G-25 (NAP-10 columns, Pharmacia). Detection of the polymorphisms was done on an Automated Laser Fluorescent Sequencer (A.L.F., Pharmacia) using Fragment Manager and the software package, Automated Linkage Preprocessor (ALP) [Mansfield et al., 1994].

Linkage Analysis

Two-point disease-to-marker analysis was conducted using the program MLINK of the LINKAGE package [Lathrop and Lalouel, 1984]. The mutation rate and gene frequency were set at 3 X 10^{-6} and 0.0001, respectively. Penetrance was set at 100% for males and either 100% or 80% for females.

RESULTS

Initially, we screened K7055 with 28 X chromosome markers spanning the chromosome. Linkage was detected to marker DXS996 ($Z=1.55$ at a $\theta=0.09$) in Xp22.3. Utilization of subsequent markers flanking this locus (DXS7100, KAL, DXS987) failed to detect linkage without recombination (Table I, column A). All analyses were conducted with penetrance set at 100% for males and females based on the observation by Stevens and Wilroy [1988] that all mothers of affected males had telecanthus. Setting penetrance at 80% for females [Robin et al., 1995], we still were unable to improve significantly our linkage results (Table I, columns A and B, markers DXS7100, DXS996, KAL, DXS987, DXS999, DXS992). However, upon testing more markers in Xp22, one of these, DXS7104, gave no recombination with a lod score of 3.3 with either penetrance function for the females (Table I, columns A and B).

TABLE I. Summary of Two-Point Disease-to-Marker Linkage Analysis for OS vs. Xp22 Markers

	A		B		C		D		E		F	
Marker	θ max	Z max	θ max	Z max	θ max	Z max	θ max	Z max	θ max	Z max	θ max	Z max
DXS7100	0.15	1.18	0.16	1.06	0.10	1.23	0.10	1.15	0.08	1.81	0.08	1.67
DXS996	0.09	1.55	0.09	1.48	0.00	2.40	0.00	2.32	0.00	2.70	0.00	2.67
DXS1283	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50
DXS1223	0.10	1.29	0.09	1.40	0.00	2.70	0.00	2.55	0.00	2.70	0.00	2.54
KAL	0.16	0.63	0.09	1.67	0.00	2.70	0.00	2.55	0.00	3.00	0.00	2.84
DXS7104	0.00	3.31	0.00	3.15	0.00	2.70	0.00	2.55	0.00	3.31	0.00	3.15
DXS1224	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50	0.00	1.50
DXS987	0.37	0.11	0.36	0.12	0.34	0.14	0.31	0.17	0.30	0.27	0.27	0.33
DXS999	0.30	0.36	0.35	0.27	0.43	0.03	0.47	0.00	0.33	0.22	0.35	0.14
DXS992	0.35	0.01	0.01	0.03	0.35	0.06	0.38	0.03	0.35	0.07	0.38	0.03

A = 100% penetrance, females as normal
B = 80% penetrance, females as normal
C = 100% penetrance, females III-3, III-6, III-7 as unknown
D = 80% penetrance, females III-3, III-6, III-7 as unknown
E = 100% penetrance, III-6 as unknown
F = 80% penetrance, III-6 as unknown

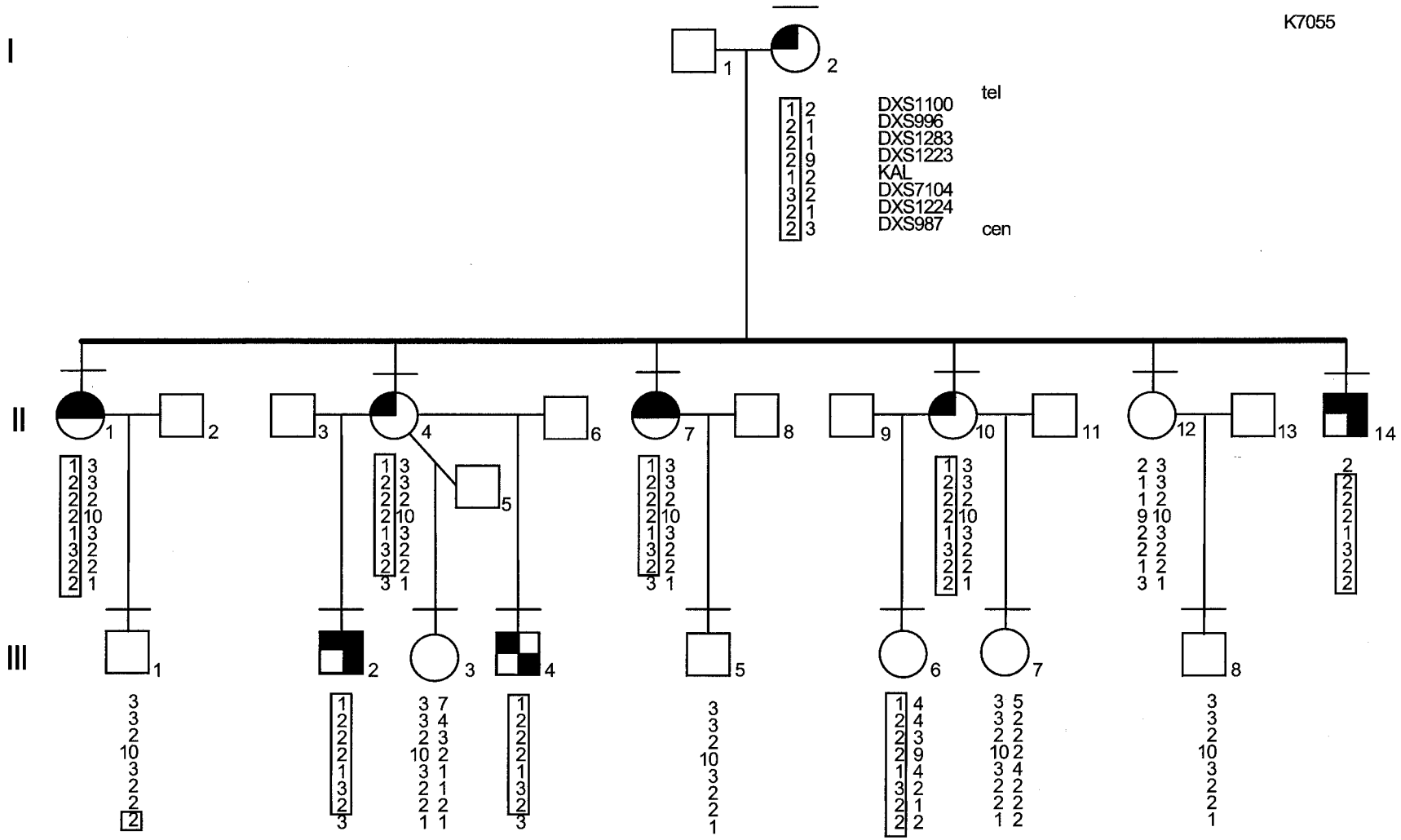

Fig. 1. Pedigree of K7055 and Xp11 haplotype. The boxed chromosome is the at risk chromosome for the Opitz GBBB syndrome.
◓ ◧ telecanthus, ◔ ◨ cleft lip/palate, ◨ hypospadias

The haplotype analysis of all the markers in Xp22 clearly indicated that person III-6 in K7055 carried the at risk genotype from DXS996 to DXS1224, but did not have telecanthus (Fig. 1). The haplotype included marker DXS7104. Close inspection of the two-point data for DXS7104 demonstrated that recombination was not being detected by computer analysis since the mother, II-10, had the same genotype as her daughter. Without paternal information, DXS7104 was uninformative relative to person III-6. The observation of a female having the at risk haplotype without clinical signs of OS had been seen by Robin et al. [1996] in two families, one of which was linked to Xp22 [Robin et al., 1995]. Therefore, we reanalyzed our data setting the affected status of females III-3, III-6, and III-7 in K7055 as unknown since these females had no offspring [Robin et al., 1995]. Linkage with no recombination at a lod score of >2 was now observed for markers DXS996, DXS1223, and KAL in addition to DXS7104 with either 100% or 80% penetrance (Table I, columns C and D) under these conditions. Setting persons III-3 and III-7 as normal and III-6 as unknown slightly increased the lod scores at DXS996 and KAL (Table I, columns E and F).

DISCUSSION

Opitz syndrome was first described as the BBB syndrome using the first letter of the last name of 3 families. Telecanthus and hypospadias were consistent findings [Opitz et al., 1969a; Christian et al., 1969]. The G syndrome included these clinical findings but in association with tracheolaryngeal abnormalities [Opitz et al., 1969b]. The G syndrome was also known as Opitz-Frías syndrome [Opitz, 1987]. These conditions are considered to be heterogeneous since some families exhibit X-linked inheritance [Stevens and Wilroy, 1988] while others appear to have autosomal dominant inheritance [Côté et al., 1981; Stoll et al., 1978; Farndon and Donnai, 1983]. Heterogeneity was confirmed recently by linkage analysis indicating one locus at Xp22 and another at 22q11.2 [Robin et al., 1995].

We now provide further linkage evidence for the existence of the Xp22 OS locus. Our linkage data localizes the OS gene between DXS987 (proximal) and DX7100 (distal), a region of about 10 cM [Nelson et al., 1995]. Combining these data with those of Robin et al. [1995] results in a SRO (smallest region of overlap) for the OS gene of about 4 cM flanked by DXS987 proximally and DXS1043 distally. The SRO contains two genes, PAPS2 (phosphoribosylpyrophosphate synthetase-II) [Taira et al., 1989] and GLRA2 (glycine receptor subunit alpha 2) [Derry and Barnard, 1991], neither of which can be viewed as good candidate genes. A search of the recently released transcript map of the human genome (http://www.ncbi.nlm.nih.gov/SCIENCE96/) revealed 11 cDNA markers localized to the region from DXS1043 to DXS1053, which extends more proximal than DXS987. One cDNA marker (SGC31598) is related to ocular albinism type I, another marker (WI13786) is highly similar to myelin proteolipid protein, and one was PRPS2. The remaining 8 were unidentified transcripts. Thus, this relatively narrow localization of OS to between DXS1043 and DXS987 should greatly facilitate the isolation and characterization of this gene which is important in midline development.

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